Microbial hydroxylation of steroids. 7. Hydroxylation of *B*-nortestosterone and related compounds by *Rhizopus arrhizus* ATCC 11145, and ^{13}C nuclear magnetic resonance spectra of some *B*-norsteroids

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The incubation of *B*-norandrost-4-ene-3-ones and *B*-nor-3β-hydroxyandrost-5-enes with *Rhizopus arrhizus* ATCC 11145 has been described. The products are consistent with a mechanism of oxidation at C-6 in which the stereochemistry of substitution at C-6 is controlled by stereoelectronic interactions in the substrate, and is not dictated by enzymic constraint during the reaction. The carbon-13 nuclear magnetic resonance spectra of several *B*-nor-Δ^4 and Δ^5 steroids have been presented.

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On décrit l'incubation des *B*-norandrostène-4 ones-3 et des *B*-norhydroxy-3β androstènes-5 avec le *Rhizopus arrhizus* ATCC 11145. Les produits sont en accord avec un mécanisme d'oxydation au niveau du carbone en position 6 dans lequel la stéréochimie de la substitution en C-6 est contrôlée par des interactions stéréoélectroniques dans le substrat et elle n'est nullement due à une contrainte enzymatique au cours de la réaction. On présente les spectres de résonance magnétique nucléaire du C-13 de plusieurs *B*-nor-Δ^4 et Δ^5 stéroides.

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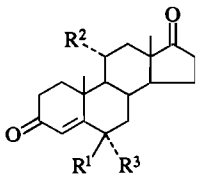
Introduction

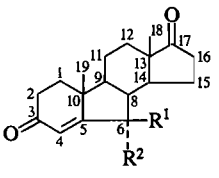
The mechanism of the hydroxylation of androst-4-ene-3,17-dione (**1***a*) at C-6β by the fungus *Rhizopus arrhizus* ATCC 11145 has been studied in some detail (1–6). The reaction proceeds by enzymic enolization of the Δ^4-3-ketone, followed by stereoelectronically controlled axial addition of electrophilic oxygen to the resulting enol, giving rise to **1***b* stereospecifically. A feature of this mechanism is that the stereochemistry of substitution at C-6 of the product is determined largely by conventional stereoelectronic interactions during the oxidation process, rather than being dictated by constraints placed upon the reactants by their interaction with the enzyme.

In this paper, the incubations with *R. arrhizus* of the Δ^4-3-keto-*B*-norsteroids **2***a* and **3***a*, and of the Δ^5-*B*-norsteroids **4***a* and **4***b* are described, which support the mechanistic interpretation outlined above. In addition, the ^{13}C nuclear magnetic resonance spectra of several *B*-norsteroids are presented.



1

a $R^1 = R^2 = R^3 = H$
b $R^1 = OH, R^2 = R^3 = H$
c $R^1 = R^3 = H, R^2 = OH$
d $R^1 = R^2 = H, R^3 = OH$



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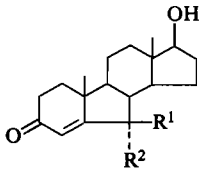
c $R^1 = H, R^2 = OH$



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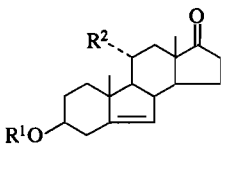
a $R^1 = R^2 = H$
b $R^1 = OH, R^2 = H$
c $R^1 = H, R^2 = OH$



4

a $R^1 = R^2 = H$
b $R^1 = Ac, R^2 = H$
c $R^1 = H, R^2 = OH$

Results and Discussion

B-norandrost-4-ene-3,17-dione (**2***a*) was metabolized by *R. arrhizus* to give three products in low yield. The difficulty of separation of these products precluded their large scale preparation, but by repeated chromatography pure samples were obtained and identified as *B*-nortestosterone (**3***a*), and the 6α- and 6β- hydroxy-*B*-norandrost-4-ene-3,17-diones, (**2***c*) and (**2***b*) respectively. The identification of **3***a* was trivial, and **2***c* has been reported previously as the product of microbial transformation of **2***a* by *Absidia orchidis* (7, 8). Confirmation of the structures of **2***b* and **2***c* rested largely on comparisons of their ^1H nmr spectral data (Table 1) with those of **1***b* and **1***c*, and, more significantly, with those of **3***b* and **3***c*. The ^1H nmr spectra of the latter two compounds, the only products of the incubation of *B*-nortestosterone (**3***a*) with *R. arrhizus*, were analysed by spin decoupling experiments.[1] In each case the C-4 hydrogen, which

[1]These experiments were performed at 250 MHz by Dr. C. Rogers of Bruker Spectrospin (Canada) Ltd.

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TABLE 1. Principal ^1H nmr signals of **1***a–c* and the *B*-norsteroids **2–7***

Compound	δ ppm					
	C-3H	C-4H	C-6H	C-11H	C-18H	C-19H
1*a*	—	5.74 (s, $W_{\frac{1}{2}}$ 3 Hz)	—	—	0.93	1.22
1*b*	—	5.84 (s, $W_{\frac{1}{2}}$ 1.8 Hz)	4.40 (t, *J* 7 Hz)	—	0.95	1.41
1*c*	—	5.77 (s, $W_{\frac{1}{2}}$ 3 Hz)	—	3.6–4.3 (m)	0.94	1.36
1*d*	—	6.20 (d, *J* 2 Hz)	4.37 (dd, *J* 5, 11 Hz)	—	0.92	1.21
2*a*	—	5.83 (t, *J* 1.5 Hz)	—	—	0.95	1.12
2*b*	—	6.00 (d, *J* 2 Hz)	4.40 (dd, *J* 2, 8 Hz)	—	0.95	1.24
2*c*	—	6.07 (d, *J* 2 Hz)	4.76 (dd, *J* 2, 7 Hz)	—	0.95	1.11
3*a*	—	5.78 (t, *J* 1.5 Hz)	—	—	0.82	1.08
3*b*	—	5.95 (d, *J* 2 Hz)	4.24 (dd, *J* 2, 8 Hz)	—	0.82	1.20
3*c*	—	6.02 (d, *J* 2 Hz)	4.60 (dd, *J* 2, 7 Hz)	—	0.81	1.10
4*a*	3.25–3.65	—	5.44 (br s)	—	0.93	0.93
4*b*	4.35–4.80	—	5.46 (d, *J* 2 Hz)	—	0.94	0.94
4*c*	3.30–3.70	—	5.41 (br s)	—	0.94	1.06
5	3.65–4.20	—	3.38 (s)	—	0.94	0.88
6*a*†	3.30–3.70	—	5.30 (d, *J* 2 Hz)	—	0.75	0.88
6*b*	4.50–4.90	—	5.46 (d, *J* 2 Hz)	—	0.83	0.92
7*a*	4.35–4.80	—	5.45 (d, *J* 2 Hz)	—	0.67	0.93

*Solvent $CDCl_3$ unless otherwise stated.
†Solvent $CDCl_3$/DMSO-d_6, 1:1.

appears in the undecoupled spectrum as a doublet, collapsed to a singlet upon irradiation at the resonance position of the hydrogen geminal to hydroxyl, thus confirming the location of the latter functional group at C-6. The chemical shifts observed for the C-19 methyl hydrogens, diagnostic of the stereochemistry of substitution at C-6 (9), are also consistent with the structures presented, as are their ^{13}C nmr data (*vide infra*).

R. arrhizus has been reported to hydroxylate 17α-methyl-*B*-nortestosterone at C-6α, C-6β, and C-11α, but without quantification or discussion of the results (10). Hydroxylation of **2***a* or **3***a* at C-11α was not observed in the present study but hydroxylation at C-6 of both substrates occurred, to give α and β isomers in approximately equal amounts in each instance. Control experiments in which **3***b* and **3***c* were used as substrate demonstrated that epimerization at C-6 does not occur under the conditions of the incubation or work-up, the C-6 alcohols being recovered unchanged in each case. The concurrent formation of both α and β alcohols at C-6 from **2***a* and **3***a* is consistent with the mechanism outlined in the Introduction: the conformation of the 5-membered B ring of *B*-norsteroids is such that both α and β positions at C-6 are stereochemically equivalent with respect to the plane of the O—C-3—C-4—C-5 enone system. This is reflected in the observed multiplicity of the C-4 hydrogen signal in the ^1H nmr spectra of **2***a* and **3***a*, appearing as a symmetrical triplet (J = 1.5 Hz) (Table 1) due to the influence of both hydrogens at C-6. Interaction of developing electron density at C-6 from a $\Delta^{3,5}$ enol of **2***a* or **3***a* with an electrophilic oxidizing species will therefore occur equally favourably on both α and β faces of the molecule. This assumes that the transition state for the reaction is product-like, an assumption which is consistent with available data on such processes (2, 11, 12).

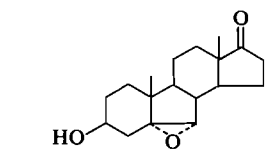
The reaction of a $\Delta^{3,5}$ enol acetate or enol ether with *m*-chloroperoxybenzoic acid has been used successfully as a model system to study the stereoelectronic control of product formation during electrophilic oxidation at C-6 in the normal steroid series (3) and related bicyclic enones (1, 3). In an attempt to use this reaction in the *B*-nor series, the preparation of compounds **8***a* and **8***b* was undertaken, using standard procedures for the preparation of $\Delta^{3,5}$ enol ethers (13) and acetates (14) from Δ^4-3-ketosteroids. Although tlc analysis indicated that product formation had occurred, all attempts at isolation resulted in reversion to the Δ^4-3-ketone. An alternative procedure, successful in converting a bicyclo [4.3.0] nonenone to its enol acetate **10**, (15), was used with *B*-nortestosterone (**3***a*) as starting material in an effort to produce the enol diacetate **9**; once again, although **9** was apparently formed in the reaction, it reverted to *B*-nortestosterone acetate on attempts at isolation. Compound **10** is reportedly unstable (15), and the extra steric strain in ring B of the steroid caused by the presence of rings C and D can account for an increased reactivity of **8***a*, **8***b*, and **9** with respect to **10**. It has therefore not been possible to pursue the model oxidations of **8***a*, **8***b*, or **9**.

TABLE 2. ^{13}C Chemical shifts of Δ^5-*B*-norsteroids **4–7**

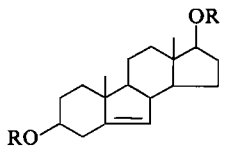
	δ ppm						
Carbon	**4***a*	**4***b*	**4***c*	**5**	**6***a*†	**6***b*	**7**
1	37.1	36.9	38.6	30.9*	37.3	37.0*	37.0
2	32.0	27.9	32.0	31.2*	31.9	28.1**	28.0
3	71.4	73.5	70.8	69.7	71.2	73.7	73.7
4	36.6	32.8	36.6*	34.6	37.3	32.8	32.8
5	150.4	149.1	150.8	69.4	150.0	148.4	148.3
6	123.4	124.3	122.0	59.2	124.8	125.5	125.8
8	45.8	45.8	42.1	42.3	46.4	45.9	46.0
9	62.6	62.6	67.6	46.3	62.9	62.5	62.3
10	44.7	44.8	45.4	38.9	45.0	44.9***	44.8
11	20.2	20.1	68.6	19.7	20.8	20.5	21.0
12	32.0	31.9	43.4	31.7	37.3	37.1*	39.3
13	49.4	49.4	50.9	49.3	46.4	44.6***	46.0
14	49.8	49.7	49.3	48.8	49.7	49.1	54.8
15	22.4	22.5	21.9	22.4	23.9	23.9	24.5
16	35.8	35.8	36.4*	35.7	30.7	27.8**	23.4
17	220.4	220.1	218.7	219.7	81.3	82.4	63.2
18	14.1	14.2	14.7	14.0	11.5	12.4	13.7
19	15.1	15.0	15.2	15.6	15.1	15.0	15.0
20							209.6
21							31.5
Other		21.4				21.2	21.4
						21.4	
		170.5				170.5	170.5
						171.1	

*Chemical shifts with asterisks may be interchanged.
†Solvent $CDCl_3$/DMSO-d_6, 1:1.

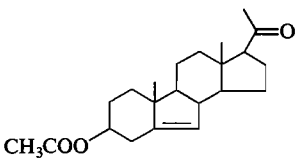
The lack of enzyme control of oxidation at C-6 is observed more strikingly in the oxidation of the Δ^5-*B*-norsteroids **4***a* and **4***b* by *R. arrhizus*. Both gave identical products, the ester function of **4***b* being hydrolyzed in conjunction with oxidative metabolism. The product of hydroxylation at C-11α, **4***c*, was identified by spectral analysis (see tables) and by comparison of physical data with those published (7, 8). The 5,6α epoxide **5** was identified by ^1H and ^{13}C nmr spectral data (Tables 1 and 2). This compound has also previously been reported as the result of the transformation of **4***a* by *Rhizopus nigricans* (7, 8).



5



6
a R = H
b R= Ac



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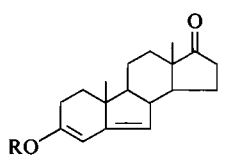
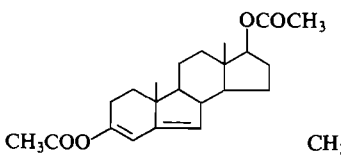


8
a R = Ac
b R = Et



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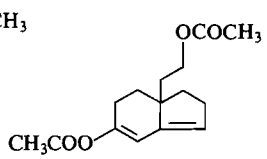

10

The stereospecific formation of the 5,6α epoxide by a micro-organism which produces the C-6β alcohol from Δ^4-3-keto substrates of the normal steroid series once again emphasizes that the stereochemistry of oxidation in this region of the molecule is controlled by the configuration and conformation of the substrate and is largely independent of the enzyme; peracid oxidation of the 5,6-double bond in *B*-norsteroids proceeds stereoselectively from the α side (16).

Product characterization in this study relied heavily upon the interpretation of carbon-13 nuclear magnetic resonance data, presented in Tables 2 and 3. The chemical shifts of the unsubstituted Δ^4-3-keto-*B*-norsteroids **2***a* and **3***a* and of the Δ^5-*B*-norsteroids **4***a*, **4***b*, **6***a*, **6***b*, and **7** were readily assigned by comparison with published data for analogous *B*-norcholestenes (Rings A and B) (17) and androstane derivatives (Rings C and D) (18). Compound **4***c* shows chemical shifts in accord with

TABLE 3. ^{13}C Chemical shifts of Δ^4-3-keto-*B*-norsteroids **2** and **3**

	δ ppm			
Carbon	**2***a*	**3***a*	**3***b*	**3***c*
1	35.3*	35.4	35.5	36.0
2	33.5	33.7	33.8	33.7
3	199.1	199.5	200.4	200.2
4	123.0	122.6	124.1	124.1
5	177.4	178.7	177.8	179.6
6	33.5	34.3	76.6	71.8
8	38.2	38.5	47.5	44.1*
9	58.5	58.5	53.3	53.7
10	43.8	43.9	42.4	43.2
11	20.1	20.5	20.3	20.3
12	31.3	36.5	36.1	36.0
13	49.2	44.8	44.7	44.7
14	51.1	50.9	49.6	44.7*
15	22.6	23.9	23.8	23.4
16	35.7*	30.6	30.8	30.6
17	219.5	81.2	81.2	81.2
18	14.3	11.5	11.4	11.4
19	17.5	17.5	18.6	17.8

*Chemical shifts with asterisks may be interchanged.

those anticipated for C-11α hydroxyl substitution (in normal steroids) (18), while **5** shows shifts, particularly those of C-1 and C-9, which confirm the α stereochemistry of the epoxide (19). The 6-hydroxy-*B*-norsteroids **2***b* and **2***c* were not obtained in sufficient quantity for acceptable ^{13}C spectra to be easily obtained, but chemical shift assignments for the analogous *B*-nortestosterone derivatives **3***b* and **3***c* are presented in Table 3.

The observed shifts at C-6, C-8, C-14, and C-19, with respect to those of the corresponding carbons of **3***a*, are characteristic of the stereochemistry of substitution at C-6 and are entirely consistent with the structures proposed.

Experimental

Apparatus, Materials, and Methods

The apparatus and techniques used were as previously described (3, 19). Analytical samples were purified and final separation of **3***b* and **3***c* performed by chromatography using a Perkin-Elmer series 3A HPLC. Incubations with *R. arrhizus* were performed as described earlier (1).

Preparation of Substrates

The following compounds were prepared by published procedures: *B*-norandrost-4-ene-3,17-dione (**2***a*) (20, 21), 3β-acetoxy-*B*-norandrost-5-ene-17-one (**4***b*) (20), *B*-norandrost-5-ene-3β-ol-17-one (**4***a*) (20), *B*-norandrost-5-ene-3β,17β-diol (**6***a*) (20), 3β,17β-diacetoxy-*B*-norandrost-5-ene (**6***b*) (20), 3β-acetoxy-*B*-norpregn-5-ene-20-one (**7**) (20). All compounds had analytical (mp) and spectral (ir) properties consistent with those reported previously (20, 21). The ^1H nmr and ^{13}C nmr data are presented in the tables.

B-*Norandrost-4-ene-17β-ol-3-one* (B-*nortestosterone*), (**3***a*)

This was prepared by the procedure used by Eder for the preparation of testosterone from androst-4-ene-3,17-dione (22). A solution of *B*-norandrost-4-ene-3,17-dione (**2***a*) (7 g) in toluene (400 mL) was flushed with N_2 and heated until 200 mL of solvent had been distilled out. The solution was then cooled to 0°C, and a solution of di-isobutyl aluminum hydride in toluene (35 mL, 25%) added by syringe over a period of 10 min. The solution was stirred at 0°C for 1 h, and then acetone (14 mL) was added, followed by 2-propanol (14 mL). The resulting mixture was allowed to stand at room temperature for 3 h, and then poured onto *N* H_2SO_4 (200 mL). The product was then extracted into ether, (4 × 200 mL), and the extract washed (saturated $NaHCO_3$, water, and brine), dried (Na_2SO_4), and evaporated. The residue (6.1 g) on crystallization from ethyl acetate afforded *B*-nortestosterone (**3***a*), (5.8 g, 83%), mp 163–165°C (lit. (21) mp 163–164°C).

Incubations with R. arrhizus *ATCC 11145*

B-*Norandrost-4-ene-3,17-dione* (2a)

Incubation of **2***a* (1 g) by the standard procedure (1) gave extracts from the medium (0.9 g) and mycelium (1.3 g). The latter contained endogenous fungal material, plus **2***a* as the only readily detectable steroid. The former extract was chromatographed on silica gel using benzene–ether gradient elution and afforded **2***a* (0.7 g) and a mixture of **3***a*, **2***b*, and **2***c* (total 0.18 g), which was separated by repeated preparative layer chromatography (Merck Silica gel 60 F_{254}, 3% methanolic chloroform) to give, in order of decreasing R_f, *B*-nortestosterone (**3***a*) (35 mg), identified by mp, tlc, and spectral comparison with an authentic sample prepared as above; 6α-hydroxy-*B*-norandrost-4-ene-3,17-dione (**2***c*) (8 mg after crystallization from ethyl acetate – hexane), mp 205–210°C (lit. (7, 8) mp 210–213°C); ir ν_{max}: 1670, 1750, 3450 cm^{-1}; *ms m/e* (%): 288 (100), 273 (35), 270 (20), 260 (15), 255 (15), 244 (40), 242 (25), 232 (60); ^1H nmr, see tables; and 6β-hydroxy-*B*-norandrost-4-ene-3,17-dione (**2***b*) (6 mg after crystallization from ethyl acetate – hexane), mp 195–198°C; ir ν_{max}: 1670, 1750, 3450 cm^{-1}; ms, M^+ calcd. for $C_{18}H_{24}O_3$: 288.172; found: 288.169; *m/e* (%): 288 (100), 273 (45), 270 (20), 260 (30), 255 (25), 244 (35), 242 (20), 232 (40); ^1H nmr, see tables.

B-*Nortestosterone* (3a)

Incubation of **3***a* (2 g) gave extracts from the mycelium (1.8 g) and medium (1.6 g). The former contained **3***a* as the only readily detectable steroid. The latter extract was chromatographed on silica gel using benzene–ether – 2% methanolic ether as eluting solvents, and gave **3***a* (0.66 g), identified by comparison with a sample of authentic material, and a mixture of **3***b* and **3***c* (total 0.81 g, ca. 1:1) separated by high pressure liquid chromatography on a column of silica gel (Whatman Partisil 20, 800 × 7.8 mm) using chloroform – ethyl acetate (40:60) as eluting solvent. Crystallization from ethyl acetate – hexane gave 6β-hydroxy-*B*-nortestosterone (**3***b*), mp 172–174°C; ir ν_{max}: 1660, 3450 cm^{-1}; ms, M^+ calcd. for $C_{18}H_{26}O_3$: 290.188; found: 290.181; *m/e* (%): 290 (100), 288 (20), 275 (50), 272 (46), 257 (57), 246 (16), 234 (70), 215 (80), 213 (70); R_f, benzene – ethyl acetate 30:70, 4 elutions, 0.5; chloroform – ethyl acetate 30:70, 4 elutions, 0.63; nmr, see tables; and 6α-hydroxy-*B*-nortestosterone (**3***c*), mp 191–193°C; ir ν_{max}: 1660, 3450 cm^{-1}; ms, M^+ calcd. for $C_{18}H_{26}O_3$: 290.188; found: 290.184; *m/e* (%): 290 (28), 288 (6), 275 (12), 272 (20), 257 (32), 246 (30), 234 (100), 215 (40), 213 (42); R_f benzene – ethyl acetate 30:70, 4 elutions, 0.42; chloroform – ethyl acetate 30:70, 4 elutions, 0.56; nmr, see tables.

3β-*Hydroxy*-B-*norandrost-5-ene-17-one* (*4a*)

Incubation of **4***a* (2.4 g) gave extracts from the medium (1.8 g) and mycelium (0.8 g). The latter contained starting material as the only readily detectable steroid (tlc) and was not examined further. The extract from the incubation medium was chromatographed on silica gel (gradient elution from benzene through ether to 5% methanolic ether) and gave **4***a* (0.57 g), identified by

comparison with authentic material, 5,6α-epoxy-3β-hydroxy-*B*-nor-5α-androstan-17-one (**5**) (0.45 g from ethyl acetate – hexane), mp 193–196°C (lit. (8) mp 195–198°C); ms *m/e* (%): 290 (100), 275 (35), 272 (66), 257 (52), 244 (46), 217 (63), 215 (95); nmr, see tables; and 3β,11α-dihydroxy-*B*-norandrost-5-ene-17-one (**4***c*) (0.49 g from ethyl acetate – hexane), mp 135–138°C (softening at 115°C) (lit. (8) mp 114–120°C (from ethyl acetate)), ms *m/e* (%): 290 (100), 275 (24), 272 (24), 257 (25), 246 (36), 231 (18), 228 (27), 215 (30), 213 (32); nmr, see tables.

3β-*Acetoxy*-B-*norandrost-5-ene-17-one* (**4***b*)

Incubation of **4***b* (1 g), and work-up as described above for the incubation of **4***a*, gave **4***b* (trace), **4***a* (trace), **4***c* (0.25 g), and **5** (0.09 g). Compounds **4***c* and **5** produced in this way were identical with samples produced by incubation of **4***a* with *R. arrhizus*.

Attempted Preparations of 3-Acetoxy- and 3-Ethoxy-B-*norandrosta-3,5-diene-17-one* (**8**a *and* **8**b), *and 3,17*β*-Diacetoxy*-B-*norandrosta-3,5-diene* (**9**)

3-Acetoxy-B-*norandrosta-3,5-diene-17-one* (**8***a*)

Perchloric acid (8 μL, 70%) was added to a solution of *B*-norandrost-4-ene-3,17-dione (**2***a*) (0.1 g) in dry ethyl acetate (20 mL) and freshly distilled acetic anhydride (2 mL). The reaction was allowed to stand at room temperature, and was monitored by tlc analysis. After a period of 2 h, when reaction was judged to be virtually complete, the mixture was poured onto 2*M* sodium bicarbonate and the product extracted into ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated to yield an oil which by ^{1}H nmr analysis was found to consist of a mixture of **2***a* (ca. 80%) and the desired product (**8***a*) (ca. 20%). All attempts at separation of this mixture by chromatography on silica gel or alumina resulted only in the recovery of **2***a*.

3-Ethoxy-B-*norandrosta-3,5-diene-17-one* (**8***b*)

B-Norandrost-4-ene-3,17-dione (**2***a*) (1.5 g) was dissolved in acetone cyanohydrin (3 mL) by gentle warming, and then aqueous ammonia (sg. 0.880, 4 drops) added to the solution. The resulting mixture was allowed to stand at room temperature for 3 h, and then filtered to yield 17ξ-cyano-17ξ-hydroxy-*B*-norandrost-4-ene-17-one (1.35 g), which was washed with ether and dried *in vacuo* to give a sample of mp 163–165°C; ir ν_{max}: 1660, 2220, 3350 cm^{-1}; ^{1}H nmr δ: 0.95 (3H, s, C-18H), 1.10 (3H, s, C-19H), 5.86 (1H, br s, C-4H); ms *m/e* (%): 299 (1), 284 (2), 272 (54), 257 (14), 274 (29), 230 (100). *Anal.* calcd. for $C_{19}H_{25}NO_2$: C 76.21, H 8.41, N 4.69%; found: C 75.98, H 8.39, N 4.59%.

The above cyanohydrin (1.1 g) was dissolved in benzene (20 mL), and then 10 mL of solvent was removed by distillation. When the solution had cooled to 65°C, freshly distilled ethyl orthoformate (2.0 mL), absolute ethanol (0.9 mL), and ethanolic HCl (80 μL of a solution of 15 mL concentrated HCl in 65 mL ethanol) were added. The resulting mixture was maintained at 65°C for 2½ h, after which time tlc analysis indicated that formation of **8***b*-cyanohydrin was essentially complete. The mixture was then neutralized by the addition of pyridine (14 μL) and evaporated *in vacuo* to yield a pale yellow solid (1.31 g), which was subsequently redissolved in 95% ethanol (20 mL) containing pyridine (2 drops). This solution was heated (steam bath) for 20 min, and then diluted with hot water and allowed to crystallize to yield 1.0 g of a solid, which by ^{1}H nmr analysis was found to consist of a mixture of **2***a* (ca. 60%) and **8***b* (ca. 40%). Attempted separation as described above gave only **2***a*.

*3*β*,17*β*-Diacetoxy*-B-*norandrosta-3,5-diene* (**9**)

Preparation of **9** was attempted using the method of Peet and Cargill (15) on *B*-nortestosterone (0.515 g). The only isolable product was *B*-nortestosterone acetate (0.47 g).

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